```
=> d l4 22 ibib ab

L4   ANSWER 22 OF 27  BIOSIS  COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER:    1973:215261  BIOSIS
DOCUMENT NUMBER:     PREV197356045226; BA56:45226
TITLE:               SIMPLE AND RAPID METHODS FOR DETECTION AND ASSAY OF
                     ACTIVITY OF THE ESCHERICHIA-COLI
                     ACYL CARRIER PROTEIN DURING ITS
                     PURIFICATION PROCESS.
AUTHOR(S):           ETEMADI A H; JOSSE M
SOURCE:              Biochimie (Paris), (1972) Vol. 54, No. 10, pp. 1349-1357.
                     CODEN: BICMBE. ISSN: 0300-9084.
DOCUMENT TYPE:       Article
FILE SEGMENT:        BA
LANGUAGE:            Unavailable


=> d his

     (FILE 'HOME' ENTERED AT 11:38:06 ON 22 JUL 2004)

     FILE 'MEDLINE, HCAPLUS, BIOSIS' ENTERED AT 11:38:32 ON 22 JUL 2004
L1          194 S ESCHERICHIA COLI ACYL CARRIER PROTEIN
L2           34 S L1 AND PURIFICATION
L3          138 DUP REM L1 (56 DUPLICATES REMOVED)
L4           27 DUP REM L2 (7 DUPLICATES REMOVED)
L5            0 S L4 AND BRADFORD

=> log
ALL L# QUERIES AND ANSWER SETS ARE DELETED AT LOGOFF
LOGOFF? (Y)/N/HOLD:y
COST IN U.S. DOLLARS                          SINCE FILE      TOTAL
                                                   ENTRY    SESSION
FULL ESTIMATED COST                                39.20      39.41

STN INTERNATIONAL LOGOFF AT 11:44:21 ON 22 JUL 2004
```

2025 Cell-Free LpxC Homogeneous Assay using a Surrogate Substrate.

W. WANG, M. MANIAR, S. LÓPEZ, C. HACKBARTH, D. CHEN, C. WU, R. JAIN, J. JACOBS, J. TRIAS, Z. YUAN. *Versicor, Fremont, CA*

UDP-3-O-(R-3-hydroxymyristoyl)-N-acetylglucosamine deacetylase (LpxC) is one of the key enzymes involved in lipid A synthesis. Because LPS is an essential cellular structure for Gram-negative bacteria, inhibitors of LpxC could be new antibiotics to treat Gram-negative infections. High throughput screening of LpxC is cumbersome since the enzyme substrate is not commercially available and difficult to prepare. In addition, current LpxC enzyme assays involve multiple separation steps and use of radiolabeled substrate, which further limits its implementation in a high-throughput mode. To address these issues, a new homogenous fluorescence-based assay was developed. The assay used the hexylamide of UDP-NAc-muramic acid as a surrogate substrate. UDP-NAc-muramic acid was obtained enzymatically and coupled to n-hexylamine. The product of the reaction, the hexylamide of UDP-NAc-muramic acid, was used as a surrogate for the natural substrate UDP-3-O-(R3-hydroxymyristoyl)-GlcNAc. LpxC catalyzed the removal of the acetyl moiety from the 2-N position of this substrate. Release of the resulting free amine was monitored by fluorescamine derivatization, thus generating a fluorescent signal. This surrogate substrate had a Km of 367 μM and Kcat of 0.36 sec-1 (compared to 2 μM and 1.5 sec-1 for the natural substrate). Higher Km values allows the use of higher substrate concentration in the assay without saturating the enzyme. As a discontinous assay, the increased substrate concentration improves significantly the signal to noise ratio. Since no separation is needed, the assay is easily adaptable to high throughput screening. This assay was used to screen chemical libraries and several novel LpxC inhibitors were identified (IC50s in the 1-10 μM range, and some active against *E. coli*).

2026 Resistance in *E. coli* to LpxC Inhibitor L-161,240 Is Due to Mutations in the *lpxC* Gene.

N. RAFANAN, S. LÓPEZ, C. HACKBARTH, M. MANIAR, P. MARGOLIS, W. WANG, Z. YUAN, R. JAIN, J. JACOBS, J. TRIAS. *Versicor, Fremont, CA*

L-161,240 is an oxazoline hydroxamic acid derivative that inhibits a key enzyme, UDP-3-O-(R-3-hydroxymyristoyl)-N-acetylglucosamine deacetylase (LpxC). LpxC is involved in the synthesis of lipid A, a component of LPS of Gram-negative bacteria. The compound is active against *E. coli* (MIC 2 μg/ml) and related bacteria. Resistant mutants have been reported, although the mechanism of resistance is not known. Resistant mutants were selected on plates containing 10 or 25 μg/ml of L-161,240 at a frequency of 10^{-9}. These mutants grew poorly and were resistant to L-161,240 (MIC 32-64 μg/ml). The susceptibility of the mutants to ciprofloxacin, gentamycin, kanamycin, ampicillin, tetracycline, erythromycin, and chloramphenicol remained unchanged. The *lpxC* gene from the wild type parent strain and from 4 resistant mutants was sequenced. Two out of four resistant strains had a single missense mutation in LpxC, resulting in an I107N substitution in the predicted protein: purified wild-type and mutated LpxC proteins were active. In addition, the wild-type and mutant *lpxC* genes were cloned and expressed in an arabinose-dependent *E. coli* strain, which has a single chromosomal copy of *lpxC* under PBAD control. In the resulting strains, in the absence of arabinose, *lpxC* is transcribed from the plasmid-based *lpxC* gene. A strain expressing the mutated *lpxC* was 16-fold more resistant to L-161,240 than an isogenic construct carrying a plasmid with a wild-type copy of *lpxC*. Both *lpxC* alleles complemented the arabinose-dependent phenotype. These experiments suggest that the mechanism of resistance in *E. coli* to L-161,240 comes from mutations in the target gene, and that the mutated target is functional but its diminished activity limits the growth rate.

2027 Protein Kinase Inhibitors as Antimycobacterial Agents.

B. WATERS[1], R. STOKES[2], D. KAU[1], S. WRIGLEY[1], J.E. DAVIES[1]. [1] *TerraGen Discovery Inc., Vancouver, BC, Canada;* [2] *Univ. of British Columbia, Vancouver, BC, Canada*

Eukaryotic-like protein kinases have been identified in many bacteria (1). They have been detected by cross-reaction with antibodies against serine/threonine and tyrosine kinases, by biochemical tests and by scrutiny of bacterial genome sequences. The functions of most of these kinases in bacteria are unknown, but in *Streptomyces* sp. some are involved in regulatory cascades affecting cell development. This finding suggested a simple generic assay for protein kinase inhibitors from which several candidate molecules have now been identified (2). The *Mycobacterium tuberculosis* genome encodes at least 12 kinases of unknown function (3). Tests of known and novel protein kinase inhibitors for growth inhibition of mycobacteria, including *M. tuberculosis*, have identified several active antimycobacterial agents. Therefore, this study suggests that evaluation of a variety of kinase inhibitors may provide novel drugs active against multi-drug resistant *M. tuberculosis* infections.

References: (1) Bakal, C.J. and Davies, J.E. Trends in Cell Biol. 10, 32-38 (2000).
(2) Waters, B. and Davies, J.E. U.S. Patent. 5,770,392 (1998).
(3) Cole, S.T. et al. Nature 393, 537-544 (1998).

2028 Discovery of a Series of Compounds that Demonstrate Potent Broad-Spectrum Antibacterial Activity and Inhibition of Era, an Essential Bacterial GTPase.

N.J. SNYDER, T.I. MEIER, C.E. WU, D.L. LETOURNEAU, G. ZHAO, M.J. TEBBE. *Eli Lilly and Company, Indianapolis, IN*

Background: An HTS (high-throughput screen) was conducted to discover inhibitors of the bacterial enzyme Era (*Escherichia coli* Ras-like protein) from *Streptococcus pneumoniae*. Era is a GTPase found in both Gram-positive and Gram-negative bacteria as well as mycoplasma. It has been shown to be essential in three different strains of bacteria including *S. pneumoniae*. The initial hits from the HTS led to the discovery of a compound, which shows broad-spectrum antibacterial activity. We describe here a preliminary SAR effort to further investigate the activities. **Methods:** Standard MCCLS protocols were followed to evaluate the activities of compounds against tested bacterial strains. **Results:** For this initial compound, MIC's against *S. pneumoniae*, *Staphylococcus aureus*, and *Moraxella catarrhalis* were 31.3, 62.5, and 31.3 mg/L, respectively. As well, this compound had good enzyme activity showing 54% inhibition at 10 mg/L. Additional screening of compounds analogous to the initial lead led to compounds with improved activity. A secondary screen was developed to test related compounds with similar pharmacophores. Compounds with up to a 1,000-fold improvement in enzyme inhibition were found with IC_{50}'s ranging from 0.01-2 mg/L. These compounds also exhibited improved broad-spectrum antibacterial activity with the best compounds displaying MIC's against *S. pneumoniae*, *S. aureus*, *Haemophilus influenzae*, and *M. catarrhalis* of 1.6, 1.6, 3.1 and 1.6 mg/L, respectively. **Conclusion:** This series of Era inhibitors could be useful new broad-spectrum antibiotics.

2029 The Use of Fluorescein-labeled Co-enzyme A for the Detection of Acyl Carrier Protein Synthase (AcpS)Activity.

K.A. MCALLISTER, J.M. RICHARDSON, G. ZHAO. *Eli Lilly and Company, Indianapolis, IN*

Acyl carrier protein synthase (AcpS) is an essential enzyme in the biosynthesis of fatty acids in bacteria. AcpS catalyzes the transfer of the phosphopantetheine group from Coenzyme A (CoA) onto apo-acyl carrier protein (apo-ACP), thereby converting it to holo-ACP. Holo-ACP plays an integral role in initiation, elongation, and translocation of fatty acids. AcpS activity has traditionally been detected by TCA precipitation of holo-ACP labeled with ^{3}H-phosphopantetheine derived from custom-synthesized ^{3}H-CoA, which is an expensive and time-consuming procedure. To replace this assay, we synthesized fluorescein-CoA, measured AcpS activity using the fluorescein-CoA, and subsequently verified the incorporation of the fluorescein-CoA into apo-ACP by mass spectrometry. When fluorescein-CoA was used as a substrate, AcpS activity was monitored by native PAGE and the incorporation of fluorescein-labeled phosphopantetheine onto apo-ACP was monitored by a fluorImager. Using this fluorescent assay, CoA was found to be competitive with respect to fluorescein-CoA and the kinetic parameters (K_m = 24 microM, V_{max}= 57 nmol/min/mg, and k_{cat}= 0.08/s) for AcpS were also determined. When the kinetic parameters of AcpS determined using fluorescein-CoA were compared with those determined using CoA, it was found that AcpS exhibited significantly higher affinity and activity towards CoA than fluorescein-CoA. The kinetic parameters determined for AcpS using PAGE were also found to be in agreement with those determined by using a HPLC method when fluorescein-CoA was used as a substrate. Although fluorescein-CoA was not as effective a substrate for AcpS as CoA, it clearly has greater convenience for the detection of AcpS activity without using radioactive CoA derivatives.

General information

www.uniprot.org	**Q7X2H4**
Entry name	**Q7X2H4**
Accession number	**Q7X2H4**
Created	TrEMBLrel. 25, 1-OCT-2003
Sequence update	TrEMBLrel. 25, 1-OCT-2003
Annotation update	TrEMBLrel. 26, 1-MAR-2004

Description and origin of the Protein

Description	Putative beta-ketoacyl synthase.
Gene name(s)	GILB
Organism source	Streptomyces griseoflavus.
Taxonomy	Bacteria; Actinobacteria; Actinobacteridae; Actinomycetales; Streptomycineae; Streptomycetacea Streptomyces.
NCBI TaxID	35619

References

[1] Fischer,C., Lipata,F., Rohr,J.,
The complete gene cluster of the antitumor agent gilvocarcin V and its implication for tl biosynthesis of the gilvocarcins.
(2003) *J. Am. Chem. Soc.* **125**:7818-7819

Position SEQUENCE FROM N.A.

Comments STRAIN=Goe 3592;

Medline 22708304

PubMed 12822997

Comments

SIMILARITY Belongs to the beta-ketoacyl-ACP synthases family.

Database cross-references

EMBL	AY233211; AAP69574.1; -.
GO	GO:0003824; F:catalytic activity; IEA. GO:0006633; P:fatty acid biosynthesis; IEA.
InterPro	IPR000794; Ketoacyl_synth.

h eb c b cg b ge

Pfam

PF00109; ketoacyl-synt; 1.

PF02801; Ketoacyl-synt_C; 1.

Keywords

Transferase;

Sequence information

Length: **391 aa**, molecular weight: **40373 Da**, CRC64 checksum: **C7939B4E92202A10**

```
SQ   Sequence   391 BP;
     MTAHITGIDI VSPLGLSREE HWKALLDGCS GLRATQSFDS SRYDNPISGE VPHFAPEGLP        60
     KRLLPATDRM TQMSLVAAAG AFDDSGVDTS RVDPLGVGVM TASTAGGYAF GQKELQNLWS       120
     KGPRYVSTHQ SYAWFYAVNT GQISIRHGCQ GHSGVIVADD AGGLDAISFA ARRLARGNRV       180
     MLTGSVDSTM CPWGRVAHTS TGMLSASTDA RAAYLPFDAR ANGWVNGEGG AHLVLQTHSD       240
     GRYAAVLGHG ATMDDPRAAP GTGLVRAIHL ALGAARLRPG DISVVFADAA GTREADTAEA       300
     AALAEVFGPD SVPVTAPKAA TGRMGCGTAA LDVATAVLAL RDQTIPPTVN VQADASLGVN       360
     LCSVATHHPL TNVLVLARGV GGFNSALIVG K                                      391
//
```

General Description References Comments Links Keywords Sequence

h eb c b cg b ge